Supporting information for

Click and Fluoresce: A Bioorthogonally Activated Smart Probe for Wash-free Fluorescent Labeling of Biomolecules

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I. Quantum yield for compound 5a-c

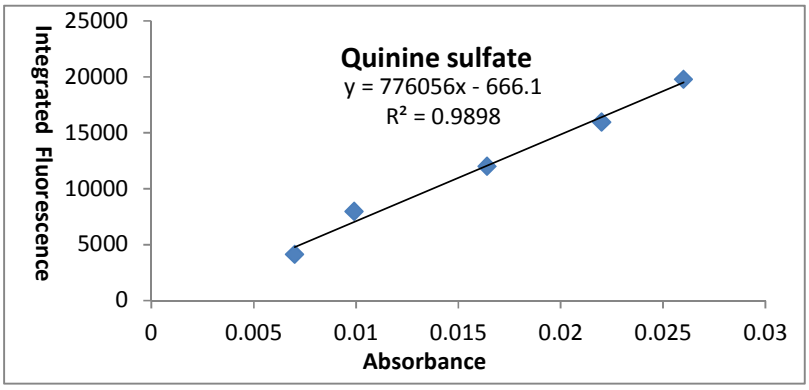

Figure S1. Plot of absorbance against integrated fluorescence intensity for the standard (excitation wavelength = 370 nM)

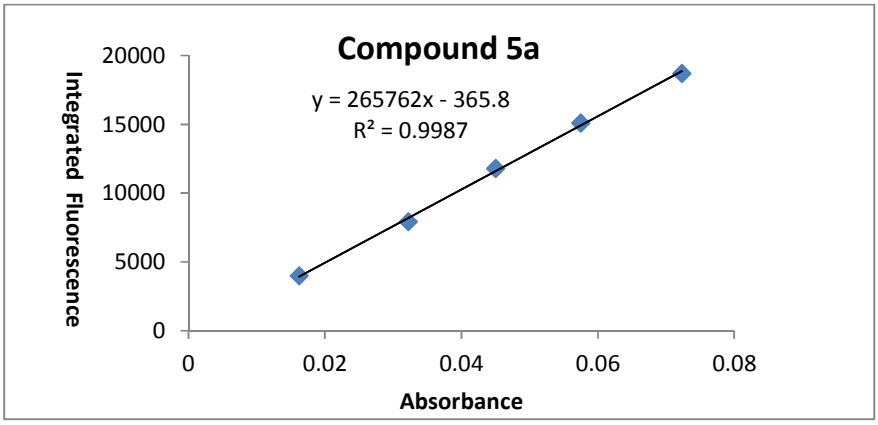

Figure S2. Plot of absorbance against integrated fluorescence intensity for **5a** (excitation wavelength = 370 nM), Φ = 0.17±0.02

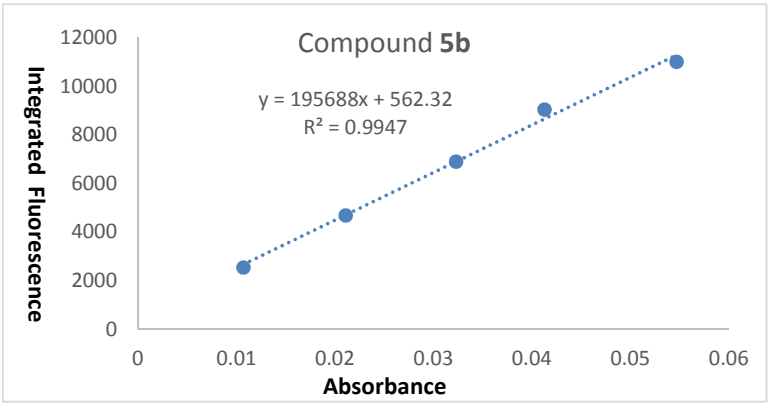

Figure S3. Plot of absorbance against integrated fluorescence intensity for **5b** (excitation wavelength = 370 nM), Φ = 0.13±0.01

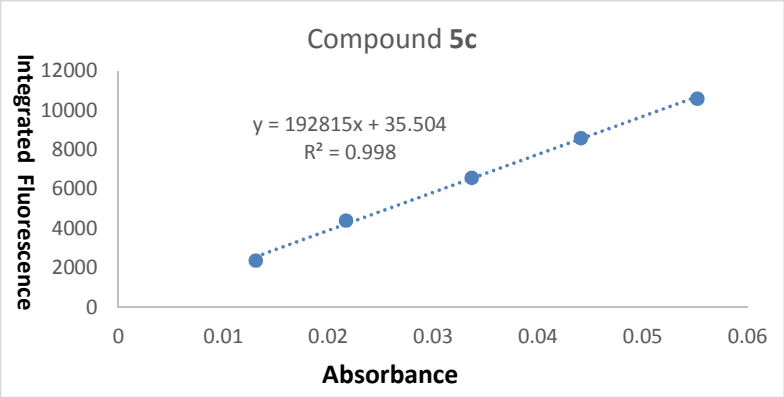

Figure S4. Plot of absorbance against integrated fluorescence intensity for **5c** (excitation wavelength = 368 nM), Φ = 0.13±0.01

II. The second order reaction rate constants between 3a-c and 4

The fluorescent property of the cycloaddition product **5a-c** greatly facilitates the determination of the second order reaction rate constants between **3a-c** and **4** (DMSO/PBS = 10:1, 37 ^{o}C, the reaction rate constant for **3c** was determined at room temperature). Briefly, the second order reaction was treated as first order reaction by using an excessive amount of **4** (>10 fold), and the obtained k' was plotted against the concentration of **4** used. The obtained slope is the second order reaction rate constant between **3a-c** and **4** (Figures S6, 8 and 10).

3.1 Determination of the reaction rate constant between 4 and 3a

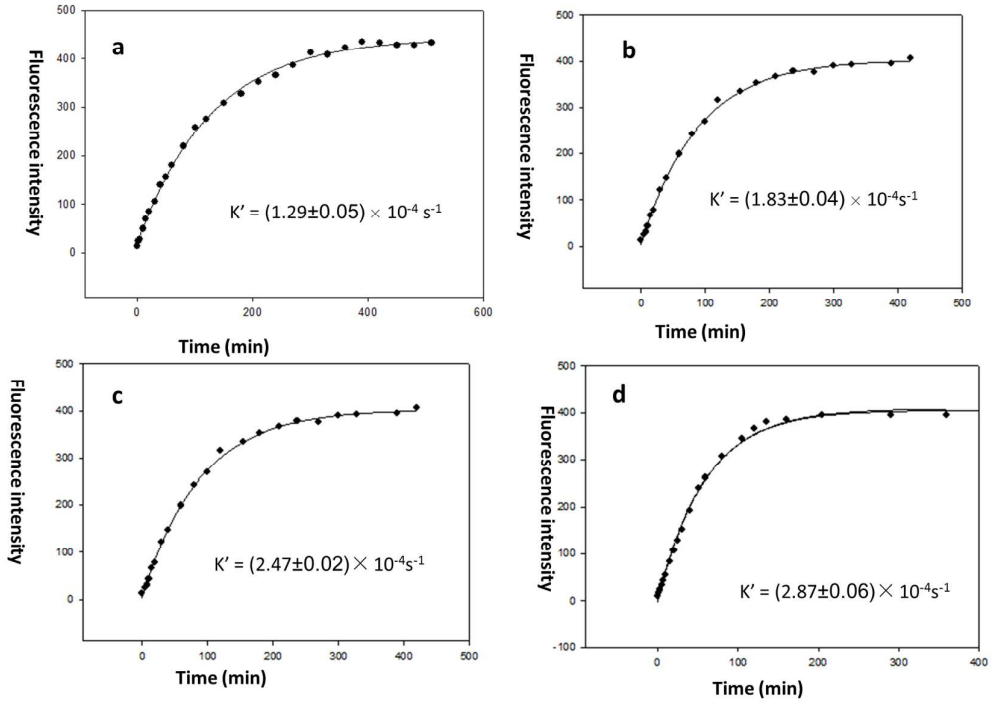

Figure S5, The pseudo first reaction between **4** and **3a**: a) **4** (1.18 mM) + **3a** (80 μM); b) **4** (1.65 mM) + **3a** (80 μM); c) **4** (2.12 mM) + **3a** (80 μM); d) **4** (2.62 mM) + **3a** (80 μM);

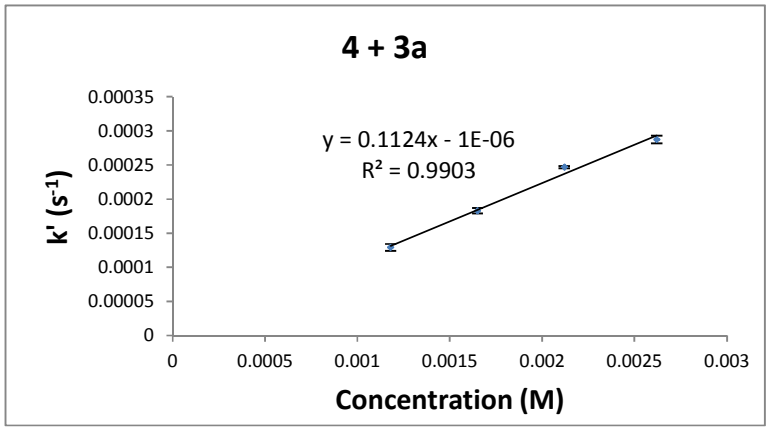

Figure S6, Plot of the obtained k' against the concentration of **4** used. The second order reaction rate constant $k = 0.11 \pm 0.01$ $M^{-1}s^{-1}$.

3.2 Determination of the reaction rate constant between 4 and 3b

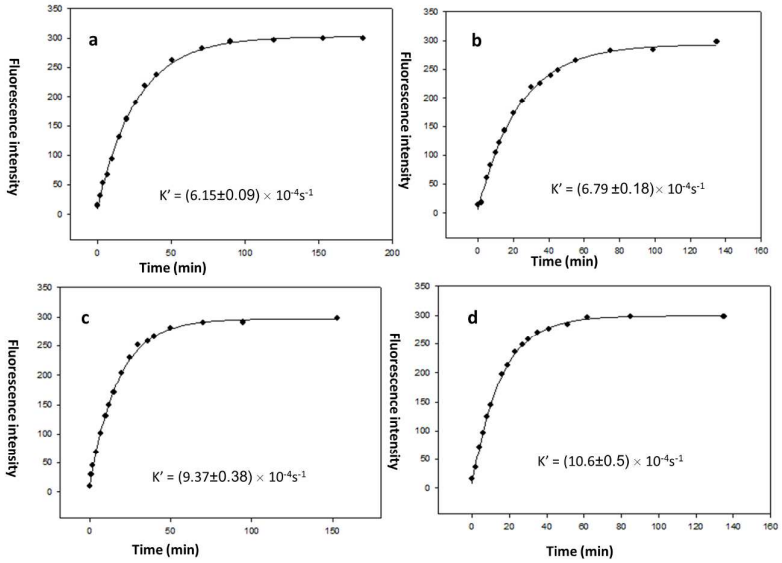

Figure S7, The pseudo first reaction between **4** and **3b**: a) **4** (492 μM) + **13b** (49 μM); b) **4** (614 μM) + **3b** (49 μM); c) **4** (738 μM) +**3b** (49 μM); d) **4** (860 μM) + **3b** (49 μM);

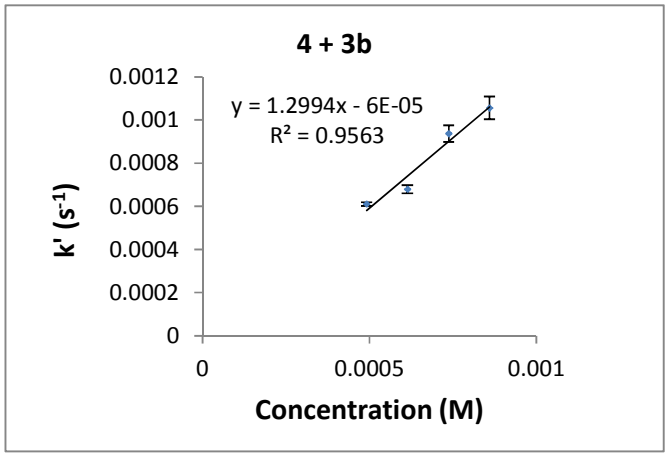

Figure S8, Plot of the obtained k' against the concentration of **4** used. The second order reaction rate constant $k = 1.30 \pm 0.11$ $M^{-1}s^{-1}$.

3.3 The determination of the reaction rate constant between 4and 3c

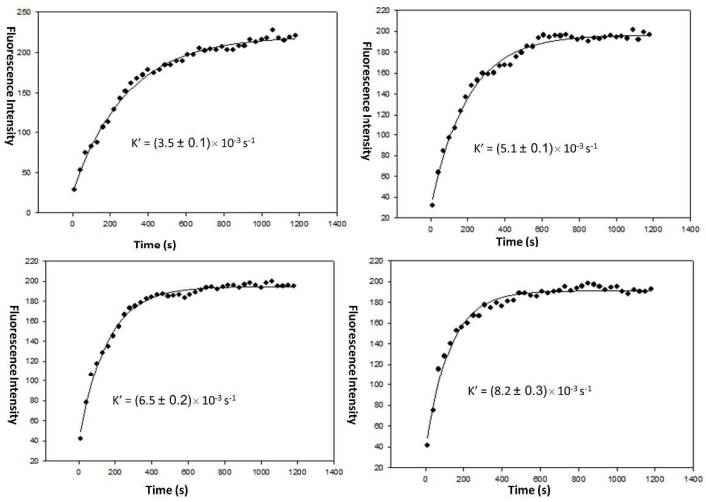

Figure S9, The pseudo first reaction between **4** and **3c**: a) **4** (498 μM) + **3c** (49 μM); b) **4** (610 μM) + **3c** (49 μM); c) **4** (747 μM) + **3c** (49 μM); d) **4** (871 μM) + **3c** (49 μM);

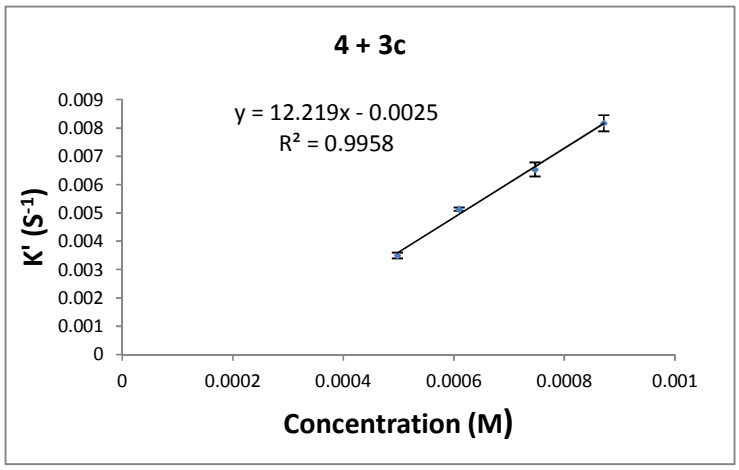

Figure S10, Plot of the obtained k' against the concentration of **4** used. The second order reaction rate constant $k = 12.2 \pm 0.6$ $M^{-1}s^{-1}$.

III. NMR spectra

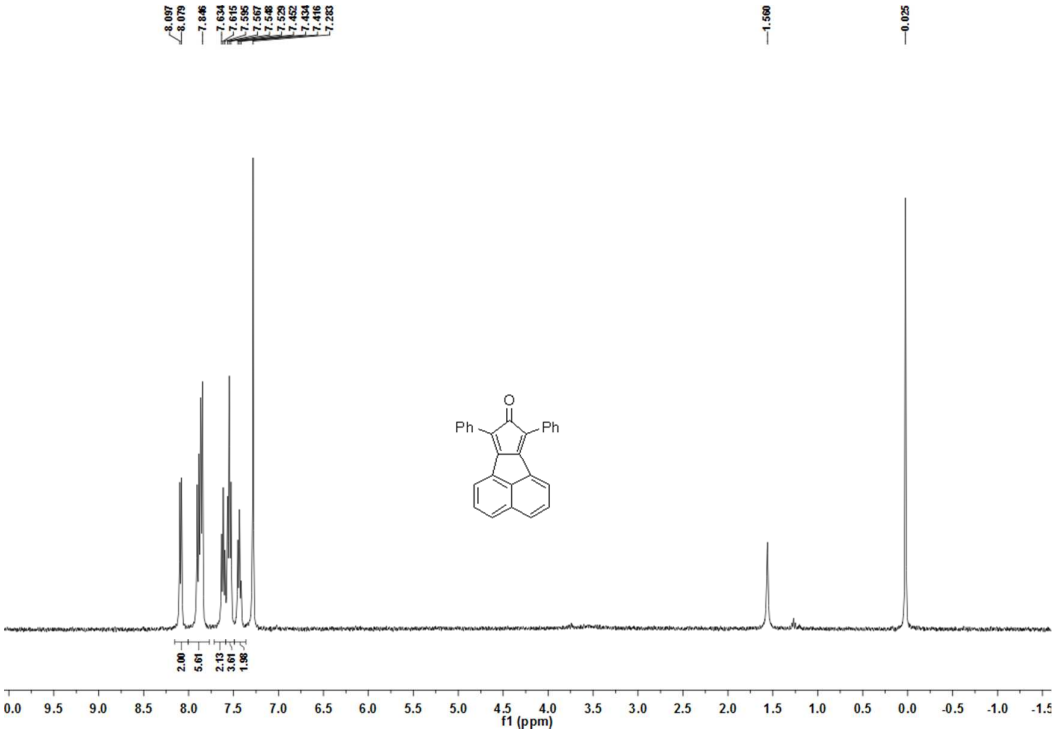

Figure S14. ^{1}H NMR spectrum for compound **3a**

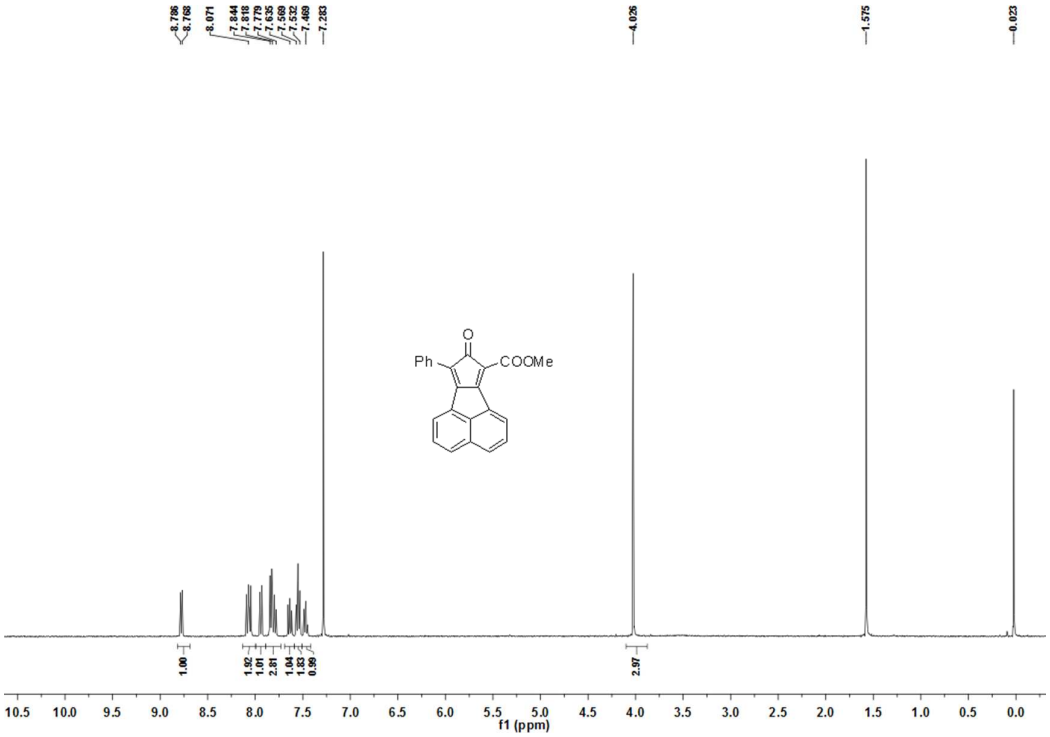

Figure S15. ^{1}H NMR spectrum for compound **3b**

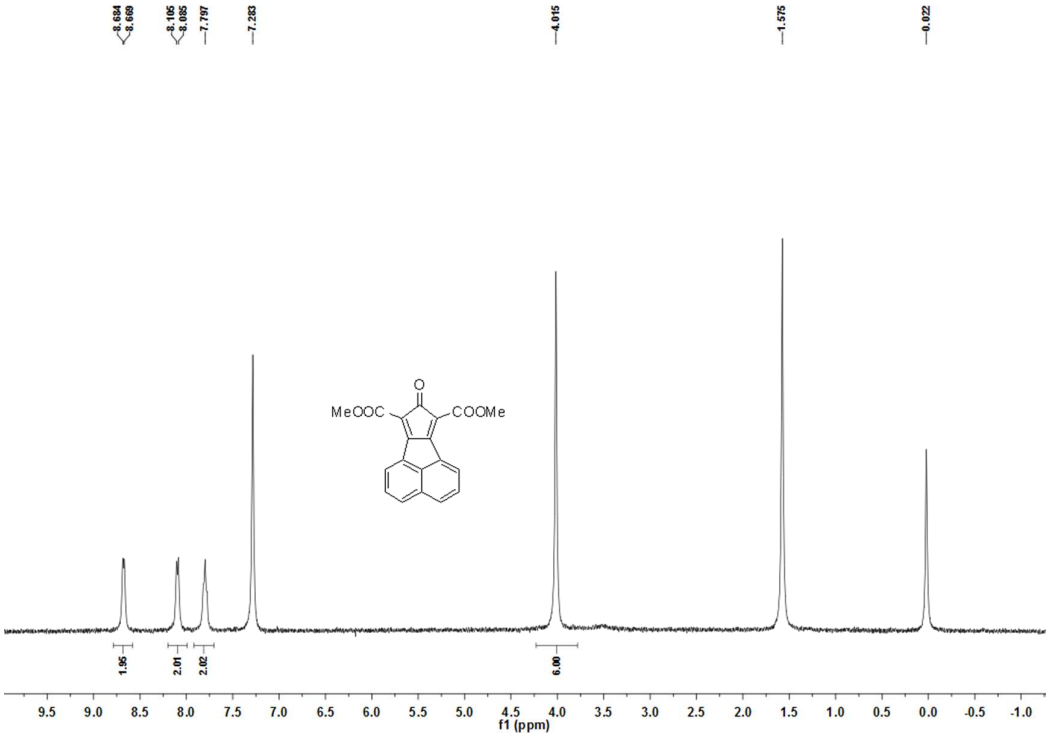

Figure S16. ^{1}H NMR spectrum for compound **3c**

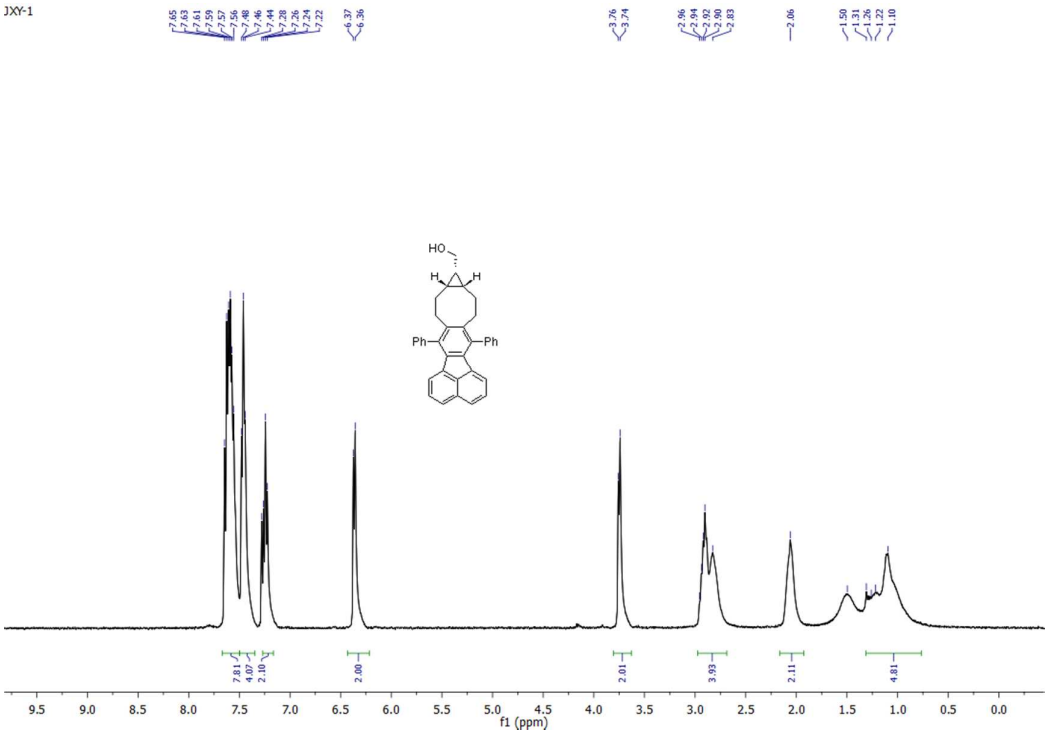

Figure S17. ^{1}H NMR spectrum for compound **5a** in $CDCl_3$ at 60 ^{o}C

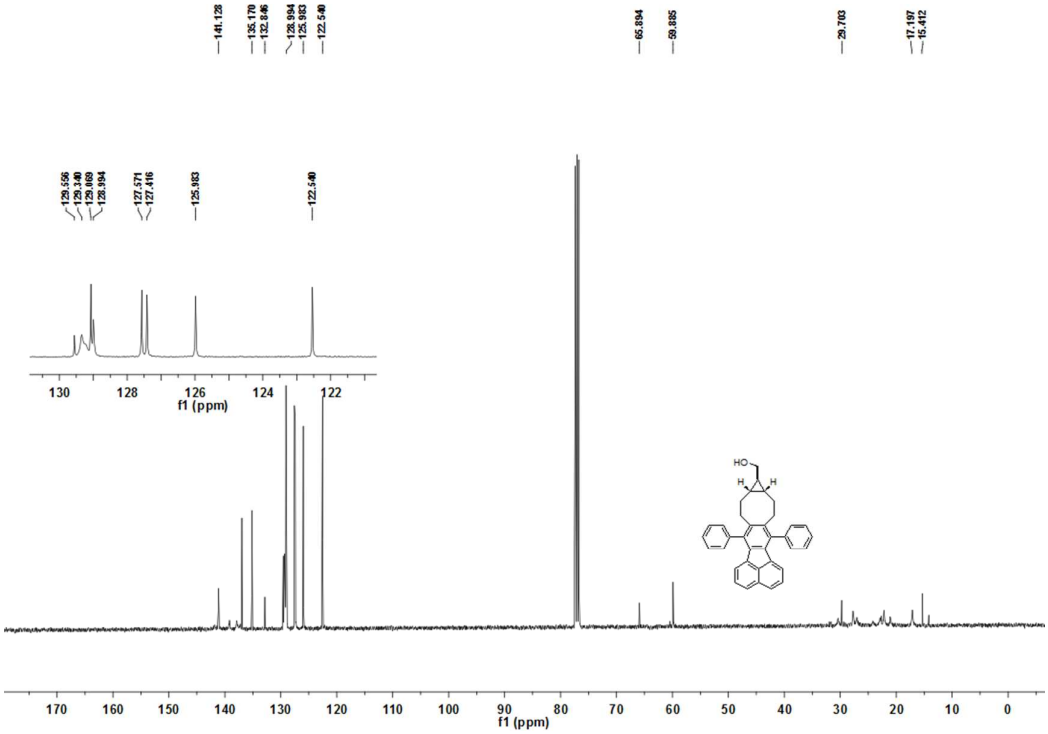

Figure S18. ^{13}C NMR spectrum for compound **5a**

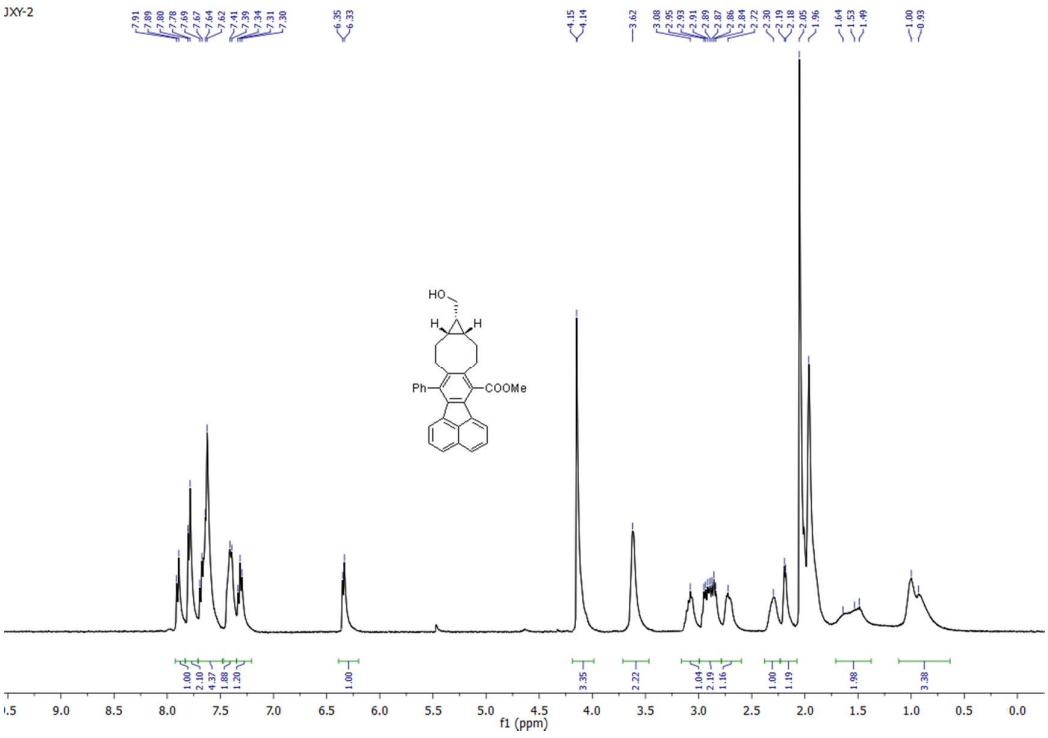

Figure S19. ^{1}H NMR spectrum for compound **5b** in CD_3CN at 60 ^{o}C

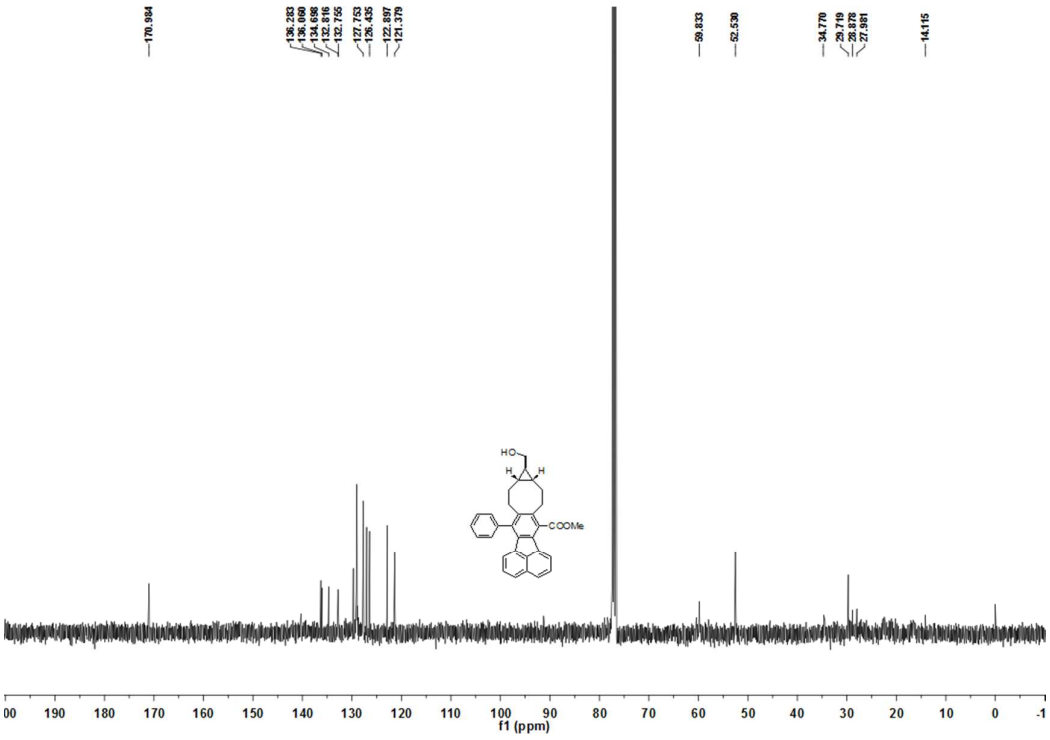

Figure S20. ^{13}C NMR spectrum for compound **5b**

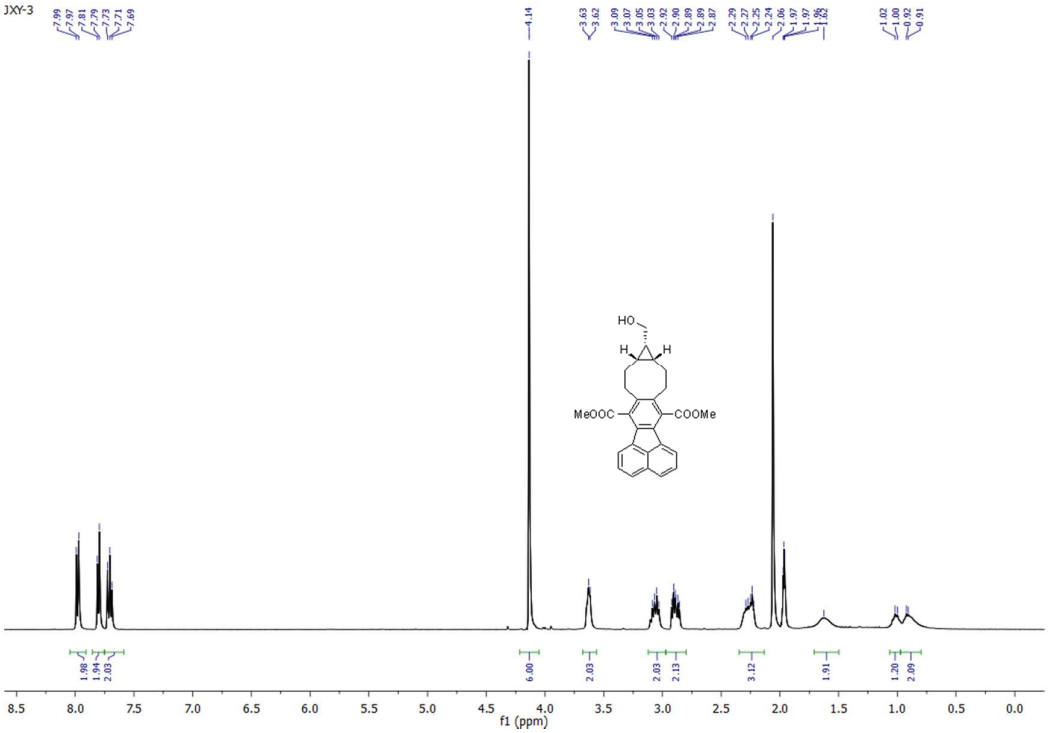

Figure S21. ^{1}H NMR spectrum for compound **5c** in CD_3CN at 60 ^{o}C

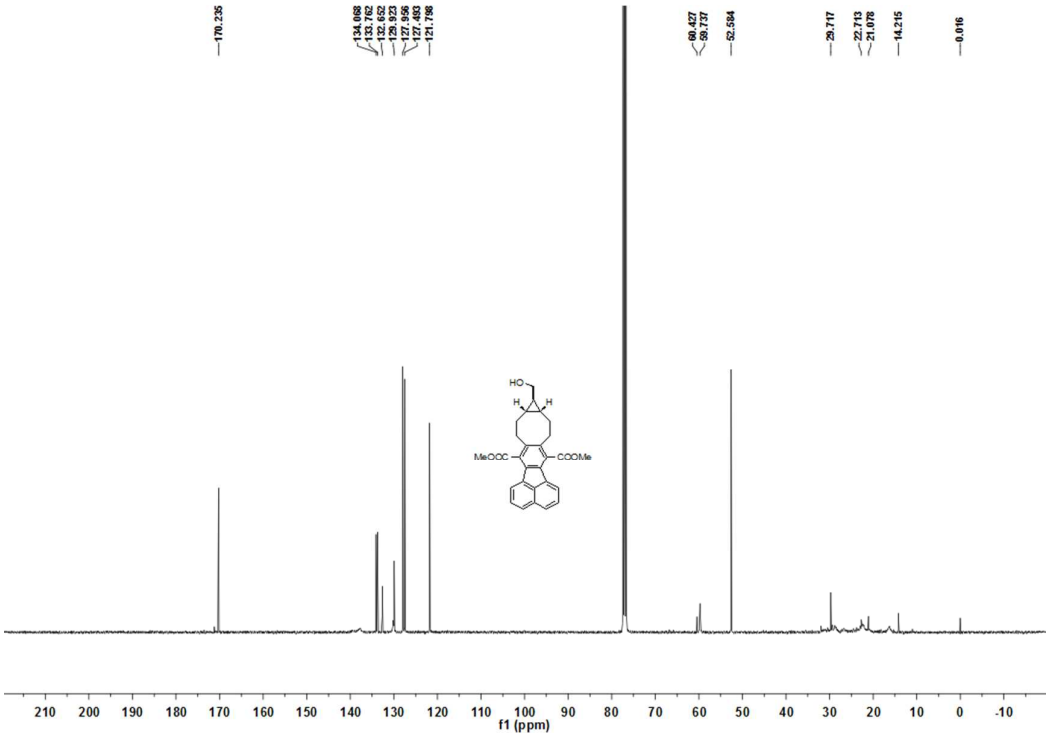

Figure S22. ^{13}C NMR spectrum for compound **5c**

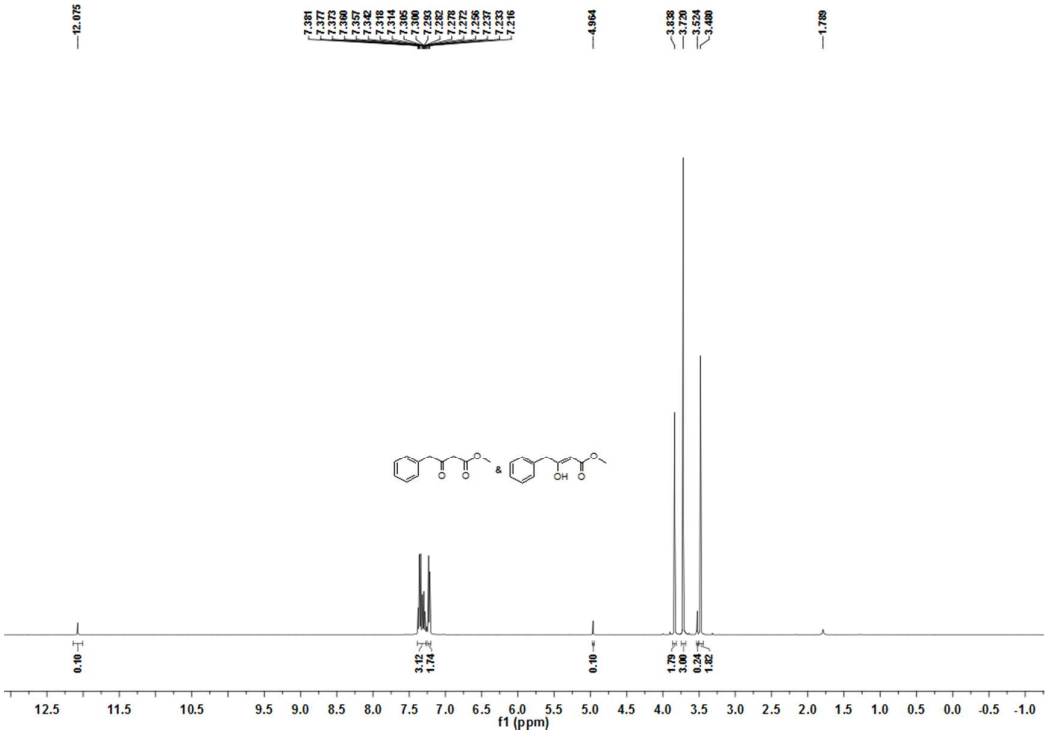

Figure S23. ^{1}H NMR spectrum for compound **1b**

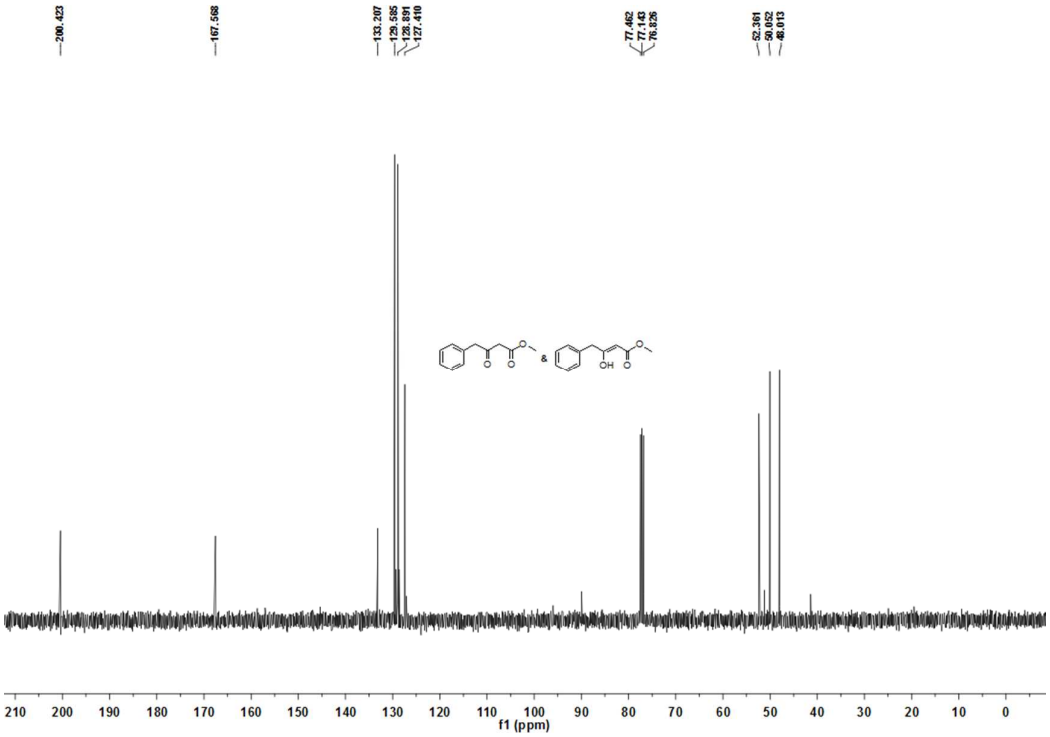

Figure S24. ^{13}C NMR spectrum for compound **1b**

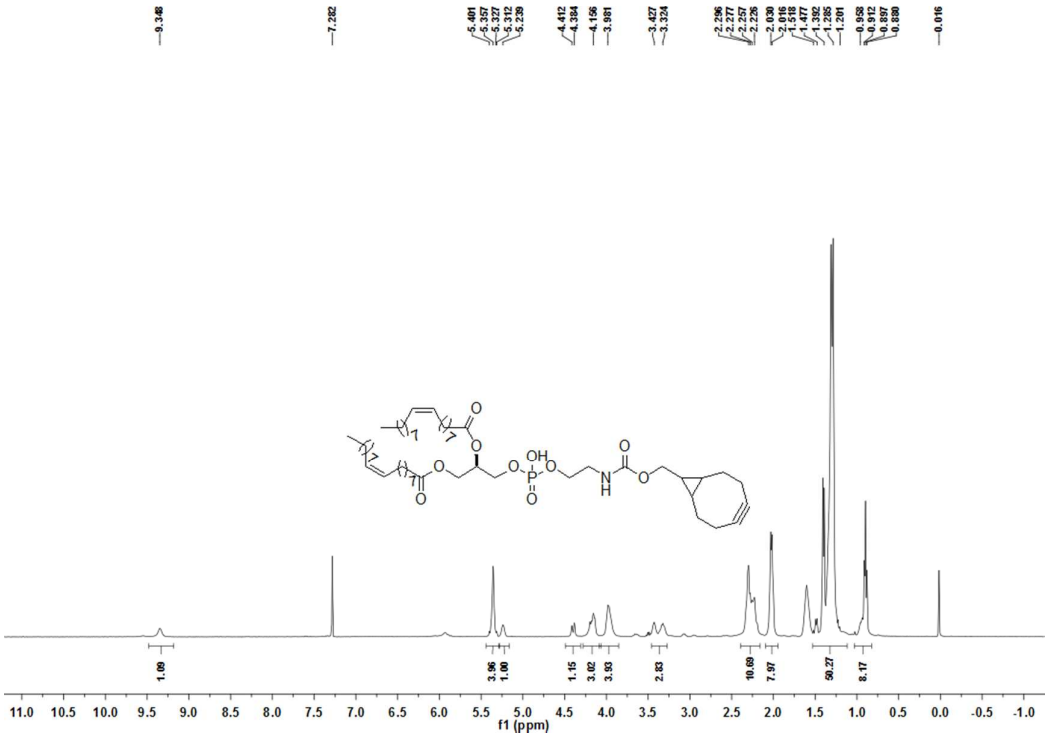

Figure S25. ^{1}H NMR spectrum for compound **7**

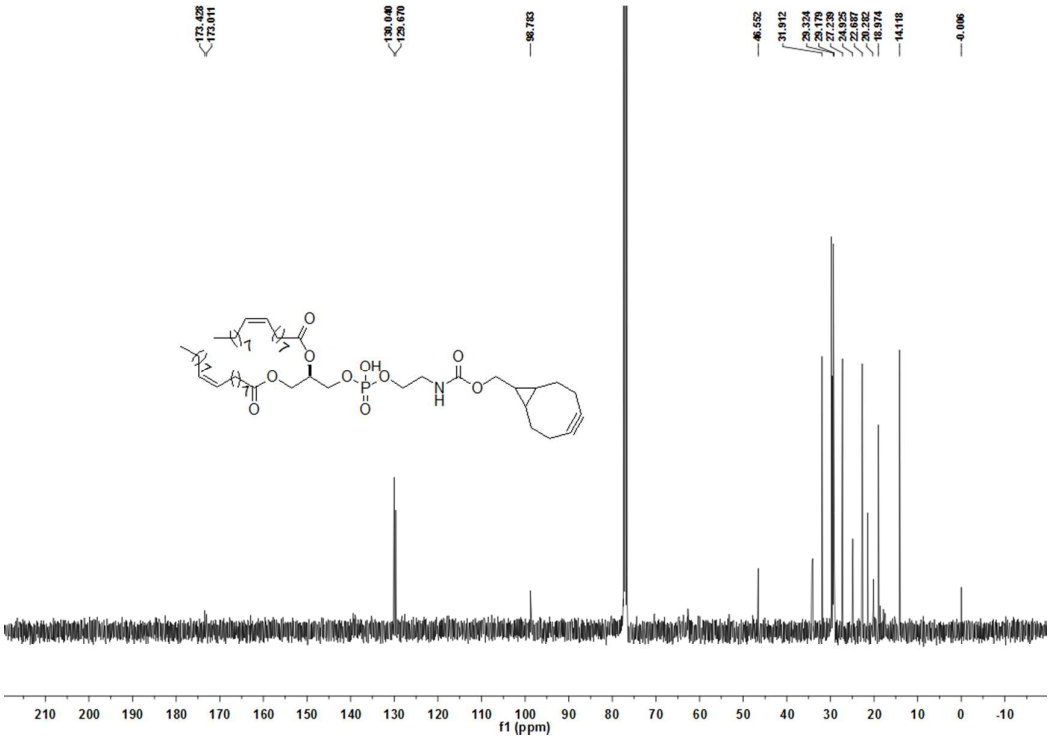

Figure S26. ^{13}C NMR spectrum for compound **7**

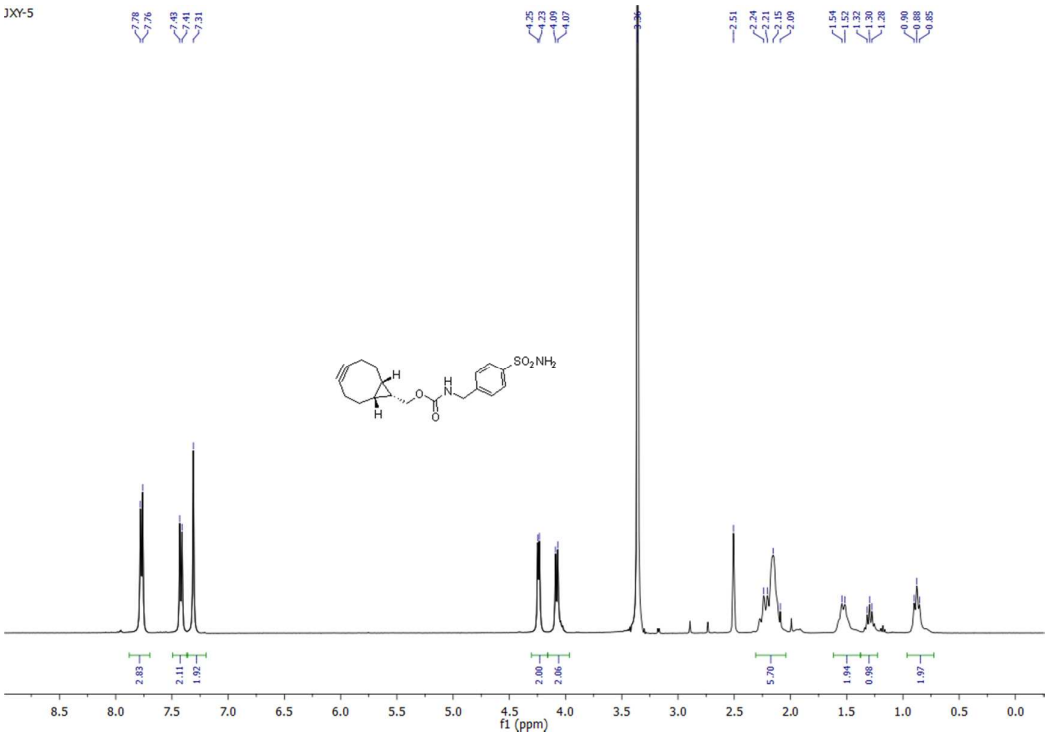

Figure S27. ^{1}H NMR spectrum for compound **9**

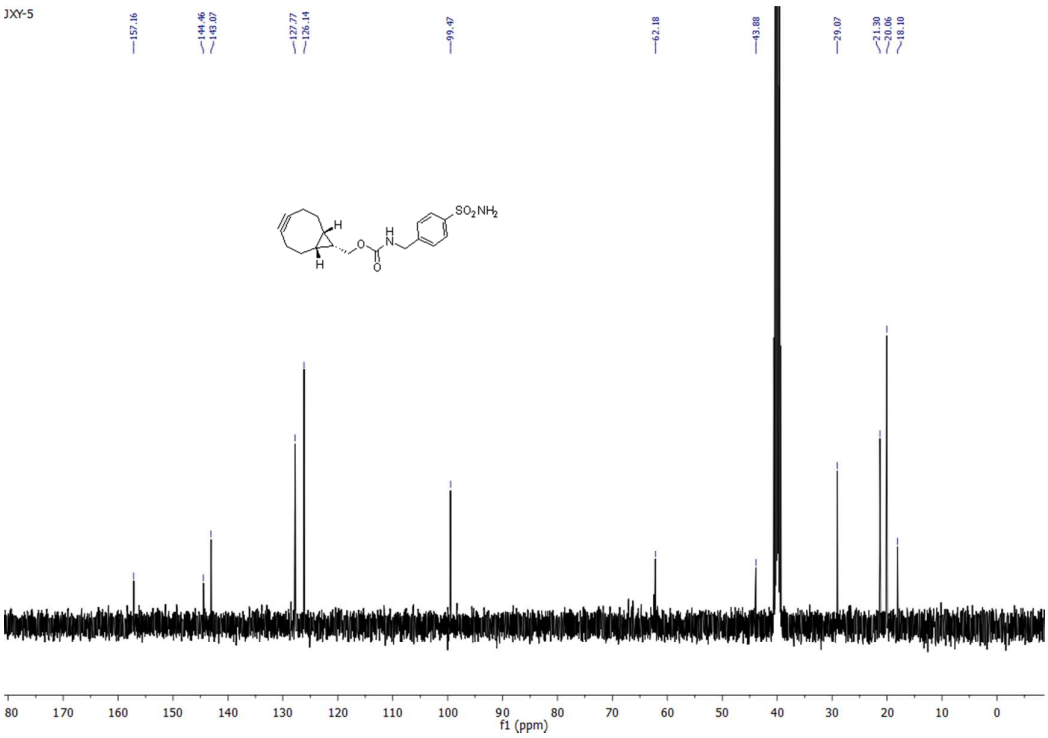

Figure S28. ^{13}C NMR spectrum for compound **9**

IV. Computational details

Table S1. Initial Cartesian coordinates (in Å) of structures by b3lyp/6-31g(d,p) method in gas phase.

	3a		
C	-4.37965622	-1.54675508	-1.97519094
C	-3.52582805	-0.69698765	-1.22814743
C	-2.18926927	-1.03464622	-1.10559769
C	-1.72736993	-2.23333441	-1.73121151
C	-2.56291672	-3.09172798	-2.46836026
C	-3.92679855	-2.70468697	-2.58401978
C	-0.34160363	-2.49408571	-1.50043858
C	0.21104092	-3.64868424	-2.02666034
C	-0.61526878	-4.52019992	-2.77956933
C	-1.95638044	-4.26109228	-3.00505759
C	-1.03661095	-0.47440465	-0.41458585
C	0.13727409	-1.40162892	-0.66546515
C	-0.67414833	0.53104073	0.42003427
C	0.77830691	0.26384144	0.76374202
C	1.23056600	-0.97345800	0.01295000
O	1.47789798	0.94464138	1.52091219
C	-1.42182231	1.68362374	0.91319065
C	-1.25250644	2.15106302	2.22964452

C	-1.97606082	3.24904364	2.68908225
C	-2.87400106	3.90729336	1.84730302
C	-3.03829896	3.46457296	0.53306143
C	-2.31826612	2.36671421	0.07023238
C	2.59536100	-1.48947700	0.05463400
C	3.69943549	-0.62018619	0.12920600
C	4.99647166	-1.12607359	0.17338200
C	5.22058175	-2.50322561	0.13581977
C	4.13452997	-3.37630020	0.04387098
C	2.83651392	-2.87503239	0.00097922
H	-5.42443138	-1.27651024	-2.06823400
H	-3.93015630	0.19010257	-0.76063322
H	-4.61389679	-3.32643770	-3.14592501
H	1.25010195	-3.90169851	-1.86755915
H	-0.17662239	-5.42170555	-3.18959832
H	-2.55254313	-4.95467900	-3.58638626
H	-0.55039269	1.65454995	2.88413692
H	-1.83706713	3.59237451	3.70689463
H	-3.43250925	4.76229952	2.20778487
H	-3.71669979	3.98307693	-0.13375313
H	-2.41963841	2.05042780	-0.96068292
H	3.53523957	0.44779704	0.14655465
H	5.83450498	-0.44274277	0.23579604
H	6.23083709	-2.89218208	0.16760033
H	4.30039406	-4.44565202	-0.00802037
H	1.99933841	-3.55332119	-0.10931125

3b

C	3.77360000	-0.12660000	-0.82730000
C	2.64850000	0.65840000	-0.44280000
C	1.43960000	0.03840000	-0.21800000
C	1.40120000	-1.49100000	-0.35030000
C	2.56400000	-2.32620000	-0.84960000
C	3.75370000	-1.54400000	-1.03470000
C	-0.03360000	-2.01920000	-0.20700000
C	-0.23220000	-3.36490000	-0.42130000
C	0.87160000	-4.17990000	-0.80570000
C	2.20590000	-3.70570000	-1.02310000
C	0.05950000	0.38510000	0.09200000
C	-0.80500000	-0.82220000	0.09850000
C	-0.67640000	1.46490000	0.36770000
C	-2.11000000	1.02790000	0.58160000
C	-2.06280000	-0.47150000	0.37810000
O	-3.06670000	1.71440000	0.85130000

C	-0.22330000	2.72060000	0.44230000
C	-1.05030000	3.78320000	0.07830000
C	-0.57750000	5.09320000	0.15600000
C	0.72220000	5.34050000	0.59770000
C	1.54920000	4.27800000	0.96180000
C	1.07640000	2.96790000	0.88400000
C	-3.11600000	-1.31310000	0.46480000
O	-4.21400000	-0.88190000	0.72510000
O	-2.94300000	-2.62440000	0.26230000
C	-4.16640000	-3.29450000	0.40300000
H	4.73040000	0.39540000	-0.97620000
H	2.74450000	1.74810000	-0.32670000
H	4.67990000	-2.04940000	-1.34580000
H	-1.23220000	-3.80590000	-0.29710000
H	0.68510000	-5.25480000	-0.94620000
H	2.98130000	-4.42180000	-1.33300000
H	-2.07530000	3.58810000	-0.27000000
H	-1.22970000	5.93120000	-0.13110000
H	1.09500000	6.37360000	0.65900000
H	2.57410000	4.47310000	1.31020000
H	1.72860000	2.12990000	1.17100000
H	-4.01490000	-4.38400000	0.23330000
H	-4.56230000	-3.13110000	1.43020000
H	-4.89280000	-2.90060000	-0.34260000

3c

C	3.90780000	0.33110000	-0.78830000
C	2.78270000	1.11610000	-0.40380000
C	1.57380000	0.49610000	-0.17900000
C	1.53540000	-1.03330000	-0.31130000
C	2.69820000	-1.86850000	-0.81060000
C	3.88790000	-1.08630000	-0.99570000
C	0.10060000	-1.56150000	-0.16800000
C	-0.09800000	-2.90720000	-0.38230000
C	1.00580000	-3.72220000	-0.76670000
C	2.34010000	-3.24800000	-0.98410000
C	0.19370000	0.84280000	0.13100000
C	-0.67080000	-0.36450000	0.13750000
C	-0.54220000	1.92260000	0.40670000
C	-1.97580000	1.48560000	0.62060000
C	-1.92860000	-0.01380000	0.41710000
O	-2.93250000	2.17210000	0.89030000
C	-0.08430000	3.19150000	0.48210000
O	1.08580000	3.42370000	0.29160000

O	-0.92690000	4.19090000	0.76760000
C	-2.98180000	-0.85540000	0.50380000
O	-4.07970000	-0.42420000	0.76410000
O	-2.80870000	-2.16670000	0.30130000
C	-4.03210000	-2.83680000	0.44190000
C	-0.23220000	5.40840000	0.79190000
H	4.86460000	0.85310000	-0.93720000
H	2.87870000	2.20580000	-0.28770000
H	4.81410000	-1.59170000	-1.30680000
H	-1.09800000	-3.34820000	-0.25810000
H	0.81930000	-4.79710000	-0.90720000
H	3.11550000	-3.96410000	-1.29400000
H	-3.88060000	-3.92630000	0.27230000
H	-4.42810000	-2.67340000	1.46920000
H	-4.75860000	-2.44290000	-0.30360000
H	-0.93900000	6.23470000	1.02980000
H	0.56310000	5.36580000	1.56930000
H	0.23040000	5.59140000	-0.20370000

4

C	-1.112219	2.049303	-0.259908
C	0.069062	1.789203	-0.137195
C	-2.526972	1.640286	-0.204921
C	1.144487	0.832246	0.178486
C	0.581208	-0.134259	1.232903
C	-2.636082	0.567319	0.890581
C	-0.537665	-1.158554	1.013077
C	-2.020681	-0.836072	0.850392
C	-1.260717	-1.517767	-0.268509
C	-0.979258	-0.918023	-1.629695
O	-2.079109	-1.136557	-2.505356
H	-3.137706	2.539474	0.043852
H	-2.859688	1.251717	-1.193042
H	1.466789	0.291860	-0.739510
H	2.014164	1.406461	0.575068
H	1.475491	-0.741239	1.530209
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H	-3.740869	0.397160	0.974515
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H	-1.877683	-0.766649	-3.368406